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Effects of nitrogen and water limitation and elevated atmospheric CO_2 on ectomycorrhiza of longleaf pine

By G. B. RUNION*[1], R. J. MITCHELL[2], H. H. ROGERS[3], S. A. PRIOR[3] and T. K. COUNTS[1]

[1] *School of Forestry, 108 M. White Smith Hall, Auburn University, Alabama 36849, USA*
[2] *Joseph W. Jones Ecological Research Center, PO Box 2324, Newton, Georgia 31770, USA*
[3] *USDA-ARS, National Soil Dynamics Laboratory, PO Box 3439, Auburn, Alabama 36831, USA*

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SUMMARY

Longleaf pine (*Pinus palustris* Mill.) seedlings were exposed to two concentrations of atmospheric CO_2 (365 or 720 μmol mol^{-1}) and two levels of N (0·02 or 0·20 mg N g^{-1} soil yr^{-1}) within open-top chambers for 20 months. Seedlings were adequately watered for 19 wk to ensure seedling establishment, after which two water-stress treatments (target values −0·5 or −1·5 MPa xylem pressure potential) were implemented. Fine-root samples were collected in July and November 1993, and in March and November 1994. Ectomycorrhizal and non-mycorrhizal short roots per unit length of fine root were quantified. The percentage of ectomycorrhizal short roots and numbers of ectomycorrhizas per unit root length were higher for seedlings grown with elevated CO_2, low N and adequate water. Interactions among main treatment variables demonstrated higher percentages of ectomycorrhizal short roots, fine root length per seedling, and total numbers of ectomycorrhizas per seedling for plants grown with high CO_2 (compared with ambient) or adequate water (compared with water stress) only under high N conditions. Increased fine-root length and ectomycorrhizal colonization under elevated CO_2 resulted in higher (almost double) numbers of ectomycorrhizas per seedling at each sampling.

Key words: Ectomycorrhizas, carbon dioxide, *Pinus palustris* (longleaf pine).

INTRODUCTION

Mycorrhiza, the symbiotic association of plant roots with fungi, represent an intimate interface between roots and rhizosphere micro-organisms which is generally beneficial to both host-plant and fungus. Mycorrhiza benefit their hosts via increased nutrient uptake, particularly of ions of low diffusivity in soil such as P, Cu and Zn (Bowen, 1973; Tinker, 1984) and by increasing mineralization and nutrient availability in some soils (Graustein, Cromack & Sollins, 1977). Mycorrhizas can increase water uptake rates of plants (Bowen, 1973; Augé, Schekel & Wample, 1987) either by providing additional water via hyphal proliferation in soil (Brownlee *et al.*, 1983) or enhancement of osmotic adjustment (Augé, Schekel & Wample, 1986). Regardless of the mechanism involved, mycorrhizas improve the drought tolerance of their host plants (Parke, Linderman & Black, 1983; Augé *et al.*, 1986, 1987), and can also contribute to plant health by protecting roots from adverse edaphic conditions such as temperature extremes, high salinity, or pathogenic micro-organisms (Marx, 1973; Duchesne, 1994).

Mycorrhizal fungi depend on their host plants for carbon, and mycorrhizas are strong sinks for host C (Paul & Kucey, 1981). In many terrestrial ecosystems mycorrhizal fungi might be the single largest consumers of net primary production (Allen, 1991). Alterations in plant C balance induced by host–mycorrhizal symbiont interactions suggest potential beneficial effects of elevated atmospheric CO_2.

The increasing concentration of CO_2 in the atmosphere (Keeling *et al.*, 1989) might have positive

* To whom correspondence should be addressed.
E-mail: runion@forestry.auburn.edu

effects on a plant species, including increased growth and yield (Rogers & Dahlman, 1993) and increased photosynthetic capacity (Long & Drake, 1992). This increased C uptake and assimilation results in increased phytomass production, the largest proportion of which is often allocated below-ground (Rogers, Runion & Krupa, 1994). Under ambient CO_2 levels, mycorrhizal infection can cause plants to assimilate more CO_2 and to allocate a greater proportion of this assimilate to their root systems (Reid, Kidd & Ekwebelam, 1983). Extra C from elevated atmospheric CO_2 can also enter the rhizosphere via root turnover and exudation (Norby *et al.*, 1987; Pregitzer *et al.*, 1995). The possibility of additional C entering rhizospheres of plants growing under atmospheric CO_2 enrichment has prompted researchers to hypothesize increased colonization by mycorrhizal fungi (Lamborg, Hardy & Paul, 1983)

Effects of CO_2 enrichment on colonization of plant roots by mycorrhizal fungi have received limited attention and results have been variable. It has been suggested that ectomycorrhizal infection might increase under higher levels of atmospheric CO_2, whereas vesicular-arbuscular mycorrhizas (VAM) might show less response, owing to a higher demand by ectomycorrhizas for plant-produced C (O'Neill, 1994). This trend is supported by much of the available literature. Ectomycorrhizal colonization increased under elevated atmospheric CO_2 on shortleaf pine (*Pinus echinata*; Norby *et al.*, 1987; O'Neill, Luxmoore & Norby, 1987), loblolly pine (*P. taeda*; Lewis, Thomas & Strain, 1994), and white oak (*Quercus alba*; O'Neill *et al.*, 1987), whereas VAM colonization was unaffected by CO_2 levels on yellow poplar (*Liriodendron tulipifera*; O'Neill, O'Neill & Norby, 1991), cotton (*Gossypium hirsutum*; Runion *et al.*, 1994), and western wheatgrass (*Pascopyrum smithii*; Monz *et al.*, 1994). However, deviations from this general pattern have been reported. Monz *et al.* (1994) showed that elevated CO_2 increased VAM colonization of blue grama (*Bouteloua gracilis*). Norby *et al.* (1986) reported that CO_2 did not significantly affect ectomycorrhizal colonization of white oak seedlings. Conroy *et al.* (1990) reported the number of root tips of a light-coloured ectomycorrhizal morphotype were higher on ambient CO_2-grown *P. radiata* seedlings under low P fertilization, but were similar under high P. However, in most of these studies where elevated CO_2 did not affect mycorrhizal density (numbers per unit root length or weight) or percentage colonization, large increases in fine-root length or weight indicated increased colonization on a whole-plant basis. Effects of atmospheric CO_2 concentration on ectomycorrhiza have also been shown to vary over time (O'Neill *et al.*, 1987). Perhaps variation in mycorrhizal response to CO_2 enrichment is due, in part, to differences in soil resource availability. We have reported that N and water limitations differ significantly in their influence on the response of longleaf pine to increasing CO_2 (Prior *et al.*, 1997); however, few studies have investigated the influence of soil resources on mycorrhizal response to a high CO_2 environment.

Conflicting reports in the literature emphasize the fact that we are only beginning to understand how below-ground processes, including colonization by mycorrhizal fungi, will be modified by rising CO_2 levels. The objective of this study was to determine the effects of elevated atmospheric CO_2 and its interactions with soil-resource limitations (N and water) on ectomycorrhizal colonization of longleaf pine seedlings.

MATERIALS AND METHODS

Plant growth and treatments

Longleaf pine (*Pinus palustris* Mill.) seedlings from a wild seed source were grown for 1 yr before being lifted from a Florida nursery in February 1993. Seedlings were stored (2 °C) for < 1 wk, graded (mean root collar diameter, 13 mm; standard deviation, 2 mm), and planted (three per container) into 192 45-l plastic containers filled with a coarse sandy medium of low fertility (Total P, K, Mg and Ca = 0·9, 5·6, 6·9 and 26·6 mg kg^{-1}, respectively). During grading, 24 randomly selected seedlings were evaluated for ectomycorrhizal infection (mean ($\pm$SD): percentage ectomycorrhizal short roots = 53·4 ($\pm$12·8); ectomycorrhizas per meter of fine root = 138·6 ($\pm$42·2); fine-root length (m) per seedling = 12·9 ($\pm$10·8); and number of ectomycorrhizas per seedling = 18·4 ($\pm$18·6) $\times 10^2$).

Seedlings were exposed to ambient (365 μmol CO_2 mol^{-1}) or elevated (720 μmol CO_2 mol^{-1}) CO_2 within an open-top chamber system similar to that described by Rogers, Heck & Heagle (1983). The chambers, CO_2 supply, and CO_2 monitoring systems have been described previously (Mitchell *et al.*, 1995). Carbon dioxide exposures were initiated on 30 March 1993. Average daytime CO_2 concentrations ($\pm$SD) through the duration of the experiment were 372·3 μmol mol^{-1} ($\pm$19·0) in the ambient chambers and 739·5 μmol mol^{-1} ($\pm$39·7) in the elevated-CO_2 chambers.

Nitrogen treatments, slightly modified from those described by Bazzaz & Miao (1993), consisted of either 0·02 or 0·20 mg N g^{-1} soil yr^{-1} applied at 3-month intervals (as sulphur-coated urea; 38-0-0). Other nutrients were maintained at non-limiting levels in all containers by application of sulphur-coated K (0-0-47 = 0·04 mg K g^{-1} soil yr^{-1}) and MicroMax® Plus (0-4-0; P = 0·14, Ca = 0·57, Mg = 0·28, and S = 0·05 mg g^{-1} soil yr^{-1}, and a complete complement of micronutrients) mixed into sand at the time containers were filled, and applied again at the end of the first year. Iron chelate (0·007 mg Fe

g^{-1} soil) was applied once in April 1993. Nitrogen treatments began at planting and CO_2 treatments were initiated on 30 March 1993.

Two water-stress treatments (Target values: −0·5 or −1·5 MPa xylem pressure potential) were implemented 19 wk after initiation of the study. Rainfall was excluded by installing a Teflon® (5-mil FEP) rain cover at the top of each chamber. At initiation of water-stress treatments, all containers were flooded with water, allowed to drain overnight, and weighed; these weights were taken as field capacity values. Gravimetric measurements, collected using a scale (Model LP-C4, 101 kg; Tri-Coastal Industries, Mukilteo, WA, USA) hung from a pneumatic lifting device of our own design, were correlated with predawn xylem pressure potentials measured on excised needles (one per treatment per chamber) at each weighing for 1 month using a pressure bomb (Scholander *et al.*, 1965). Containers received enough water to return them to field capacity weight when gravimetric measurements indicated the appropriate stress level had been achieved; xylem pressure potentials continued to be taken at least once a month as a check on gravimetric readings. New field capacity values were determined after the November 1993 harvest and midway through the second growing season (July 1994) to compensate for changes in plant mass; xylem pressure potential was measured at each weighing for 1 month following determining of new field capacity weights. Average xylem pressure potentials of needles immediately before watering were −0·6 and −1·3 MPa for seedlings in the adequately watered and water-stressed treatments, respectively. Containers in the adequately watered treatment received water every 3–4 d and those in the water-stress treatment every 12–20 d, depending upon differences in evaporative demand between seasons. Deionized water was used to ensure that fertility treatments remained unaffected by watering treatments. Soil pH increased during the course of the experiment in all treatments (initial pH = 5·1). At the final harvest, pH was unaffected by CO_2 (ambient, 6·2; elevated, 6·3) or water (adequate water, 6·3; water stress, 6·2), but was slightly higher under low than high N (6·5 and 6·1, respectively). In all cases, soil pH remained below that reported to reduce ectomycorrhizal development (Marx, 1990)

Treatments were arranged in a split-plot design with six replications. Carbon dioxide treatments (main plots) were randomly assigned to chambers (total = 12). Nitrogen and water-stress treatments (subplots) were randomly assigned, in a 2×2 factorial design, to a total of 16 containers within each chamber; container locations were re-randomized monthly.

Plant harvests and ectomycorrhizal assessment

One container from each treatment in each chamber was destructively harvested in July 1993, November 1993, March 1994 and November 1994, corresponding to 4, 8, 12 and 20 months of CO_2 exposure. Since water-stress treatments were imposed following the first harvest, data from two containers for each chamber were combined for each N treatment at this harvest. At each harvest, plants were carefully extracted from soil to prevent damage to the root system; the coarse sandy medium facilitated this extraction process, thereby minimizing damage to roots. Plants were separated into needles, stems, taproots, lateral roots and fine roots, and f. wt recorded. Twenty to 30 segments, each 3–8 cm long, were removed from fine roots collected from the interior portion of the root system of each seedling and stored in a solution of glycerine and sterile water (50:50 (v/v) for quantification of ectomycorrhizas. Fine-root length was measured on a subsample from the total mass of fine roots using a Comair Root Length Scanner (Hawker de Havilland, Port Melbourne, Australia). These fine roots were then oven-dried (55 °C) to a constant weight, and the d. wt of the subsample was recorded. Total fine-root length per seedling was extrapolated based on fresh to dry weight ratios for each subsample.

Numbers of ectomycorrhiza, non-mycorrhizal short roots, and fine-root length (cm) were quantified (Grand & Harvey, 1982), using a dissecting microscope, for each of 10 segments selected from the total stored based on low numbers of broken short roots. Ectomycorrhiza were quantified by morphotype (Meier, 1991), determined on the basis of colour and shape, and included brown, tan, white and coralloid types. The percentage of ectomycorrhizal short roots and numbers of ectomycorrhiza m^{-1} fine root were calculated. Total numbers of ectomycorrhizas per seedling were calculated from ectomycorrhizas m^{-1} fine root and total fine-root length.

Tissue analyses

Plant tissues were freeze-dried, ground to pass through a 0·2-mm mesh sieve, and N concentrations were determined using a LECO 600-CHN analyser (LECO Corp., St Joseph, MI, USA). Soluble sugars and starch were determined for each tissue using procedures described by Tissue & Wright (1995). Ground plant material was treated with a solution of methanol:chloroform:water (12:5:3 (v/v)) to separate the soluble sugars from the pellet fraction. The pellet was then treated with perchloric acid (35% (v/v)) to hydrolyse the starch, and soluble sugar and starch concentrations were determined colorimetrically using phenol–sulphuric acid.

Data analysis

Data were totalled for each seedling and averaged for each treatment in each chamber before analysis. Analysis was conducted using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS, 1985). Error terms appropriate to the split-plot design were used to test the significance of main effects and their interactions.

RESULTS

Morphotype responses

All ectomycorrhizal morphotypes responded similarly to main treatment variables (e.g. numbers per unit length of fine root were higher under elevated CO_2, low N fertility and adequate water); therefore, counts totalled across all morphotypes are presented (Tables 1 and 2). Two exceptions to this general pattern were noted (data not shown): there were fewer ($P = 0{\cdot}07$) coralloid ectomycorrhiza at elevated CO_2 at the 20-month harvest, and the white morphotype was less frequent ($P = 0{\cdot}02$) at low N fertility at the 8-month harvest.

Main treatment effects

The percentage of short roots which were ectomycorrhizal and the total number of ectomycorrhizas per unit length of fine root were generally higher at elevated CO_2 (Table 1). Total fine-root length was greater at elevated CO_2 except at the final harvest (Table 1). Due to increases in total fine-root length and in mycorrhizal colonization, elevated CO_2 resulted in higher numbers of ectomycorrhiza per seedling at each harvest.

The percentage of ectomycorrhizal short roots, and the number of ectomycorrhizas per unit fine-root length were significantly higher at low N fertility and at adequate water throughout the study; the number of ectomycorrhizas per unit length was not affected by water treatment at the final harvest (Table 1). Fine-root length and the number of ectomycorrhizas per seedling were higher with adequate water. Fine-root length was greater at high N fertility. However, due to large differences in colonization, number of ectomycorrhizas per seedling were higher at low N at the first harvest; thereafter, large differences in fine-root length

Table 1. *Main treatment effects for longleaf pine mycorrhizal variables for seedlings harvested at four periods following initiation of CO_2 exposure*

Variable† sample date‡	CO_2 concentration (μmol mol^{-1})			N fertility regime (mg g^{-1} soil yr^{-1})			H_2O stress regime (MPa)		
	365	720	$P > F$§	0·02	0·20	$P > F$	−1·5	−0·5	$P > F$
% Myc									
July 93 (4)	27·6	38·0	0·07	49·2	16·4	< 0·01	—	—	—
Nov. 93 (8)	41·4	53·2	< 0·01	54·4	40·3	< 0·01	41·5	53·1	< 0·01
Mar. 94 (12)	41·1	50·3	0·16	51·7	39·7	0·01	40·3	51·0	0·02
Nov. 94 (20)	29·6	38·5	0·04	43·3	24·9	< 0·01	31·5	36·7	0·09
Mycs/m									
July 93 (4)	85·9	131·2	< 0·01	169·2	48·0	< 0·01	—	—	—
Nov. 93 (8)	159·0	216·5	0·04	222·4	153·2	< 0·01	155·9	219·6	< 0·01
Mar. 94 (12)	160·0	201·6	0·23	212·1	149·5	0·01	154·8	206·8	0·03
Nov. 94 (20)	109·2	127·6	0·21	148·5	88·3	< 0·01	114·0	122·8	0·55
Fine root/tree (m)									
July 93 (4)	98·2	172·6	< 0·01	118·3	152·5	0·09	—	—	—
Nov. 93 (8)	236·0	331·3	0·02	209·3	358·0	< 0·01	236·8	330·5	< 0·01
Mar. 94 (12)	270·8	351·5	0·02	197·4	424·9	< 0·01	246·5	375·7	< 0·01
Nov. 94 (20)	613·0	718·7	0·24	388·0	943·7	< 0·01	545·4	786·3	< 0·01
Mycs/tree									
July 93 (4)	88·9	190·8	< 0·01	202·7	77·0	< 0·01	—	—	—
Nov. 93 (8)	353·9	722·7	< 0·01	461·4	615·2	0·05	360·2	716·3	< 0·01
Mar. 94 (12)	436·2	704·3	0·10	441·1	699·4	0·03	332·7	807·8	< 0·01
Nov. 94 (20)	568·7	817·3	0·03	545·0	841·0	0·02	559·2	826·7	0·04

† Variables: % myc, % of short roots which were ectomycorrhizal; mycs/m, number of ectomycorrhizas per metre of fine root; fine root/tree, total fine root length (m) per seedling; mycs/tree, number (×100) of ectomycorrhizas per seedling.

‡ Values in parentheses, following sample date, are months of exposure to CO_2 and N treatments.

§ Probability of a greater F value calculated, using appropriate error terms for the split-plot design, under GLM. Water-stress treatments were initiated after the first harvest.

Table 2. *Interactions among selected main treatment effects for longleaf pine mycorrhizal variables for seedlings harvested at four periods following initiation of CO_2 exposure*

Variable† sample date‡	N fertility regime (mg g^{-1} soil yr^{-1})	CO_2 concentration (μmol mol^{-1})		H_2O stress regime (MPa)	
		365	720	−1·5	−0·5
% Myc					
July 93 (4)	0·02	41·8	56·5	—	—
	0·20	13·4	19·4	—	—
Nov. 93 (8)	0·02	52·3	56·4	52·1	56·6
	0·20	30·5	50·1**§	31·0	49·6**
Mar. 94 (12)	0·02	49·6	53·8	49·9	53·5
	0·20	32·5	46·8	30·7	48·6**
Nov. 94 (20)	0·02	41·4	45·1	42·8	43·8
	0·20	17·8	31·9**	20·2	29·6*
Fine root/tree (m)					
July 93 (4)	0·02	107·3	129·2	—	—
	0·20	89·0	216·0**	—	—
Nov. 93 (8)	0·02	178·5	240·0*	205·5	213·1
	0·20	293·6	422·5**	268·1	447·9**
Mar. 94 (12)	0·02	178·4	216·4	175·4	219·4
	0·20	363·2	486·5	317·7	532·0**
Nov. 94 (20)	0·02	393·8	382·2	371·7	404·3
	0·20	832·2	1055·2**	719·1	1168·3**
Mycs/tree					
July 93 (4)	0·02	143·1	262·3	—	—
	0·20	34·7	119·2	—	—
Nov. 93 (8)	0·02	370·6	552·2	412·1	510·7
	0·20	337·2	893·2**	308·4	922·0**
Mar. 94 (12)	0·02	381·1	501·0	359·6	522·5
	0·20	491·3	907·6*	305·7	1093·1*
Nov. 94 (20)	0·02	554·4	535·6	530·9	559·1
	0·20	583·0	1099·0**	587·6	1094·4**

† Variables: % myc, % of short roots which were ectomycorrhizal; fine root/tree, total fine root length (m) per seedling; mycs/tree, number (×100) of ectomycorrhizas per seedling.

‡ Values in parentheses, following sample date, are months of exposure to CO_2 and N treatments.

§ Probability of a greater F value for differences between CO_2 or water stress treatments at a given level of N calculated using contrasts under GLM: * and ** imply significance at $0{\cdot}01 > P < 0{\cdot}10$ and $P < 0{\cdot}01$, respectively.

Water-stress treatments were initiated after the first harvest.

resulted in number of ectomycorrhizas per seedling being higher at high N (Table 1).

In addition to mycorrhizal development, main treatments strongly influenced tissue N levels. Foliar N concentrations were significantly lower for plants grown at elevated CO_2, low N and with adequate water throughout the study (Fig. 1); N concentrations of other plant tissues were similarly affected (data not shown). Tissue N concentrations were negatively correlated with the percentage of ectomycorrhizal short roots and the number of ectomycorrhiza m^{-1} fine root at all harvests (Pearson Correlation Coefficients ranged from −0·45 to −0·82, for the various harvests, with associated significance levels always $< 0{\cdot}01$). Significant negative correlations were also observed for the percentage of ectomycorrhizal short roots and number of ectomycorrhiza m^{-1} fine root with root soluble-sugar concentration at the 4-, 8-, and 20-month harvests (Pearson Correlation Coefficients ranged from −0·24 to −0·59, with significance values $< 0{\cdot}01$ at 4 and 20 months, and $< 0{\cdot}10$ at the 8-month harvest). However, percent ectomycorrhizal short roots and number of ectomycorrhiza m^{-1} fine root were positively correlated with root starch concentration at all but the final harvest (Pearson Correlation Coefficients ranged from 0·39 to 0·63, with significance values always $< 0{\cdot}01$).

Interactions among main effects

No significant interactions were observed for the number of ectomycorrhiza per unit root length. Carbon dioxide × N interactions occurred for the percentage of ectomycorrhizal short roots (8 and 20 months), fine root length per seedling (4 and 8 months), and the number of ectomycorrhiza per seedling (8 and 20 months). In general, values were higher for seedlings at elevated CO_2 only at high N fertility (Table 2). Water × N interactions were observed for the percentage of ectomycorrhizal short roots (8 months), fine-root length per seedling (all harvests), and number of ectomycorrhizas per seedling (all harvests). In all cases, adequately watered

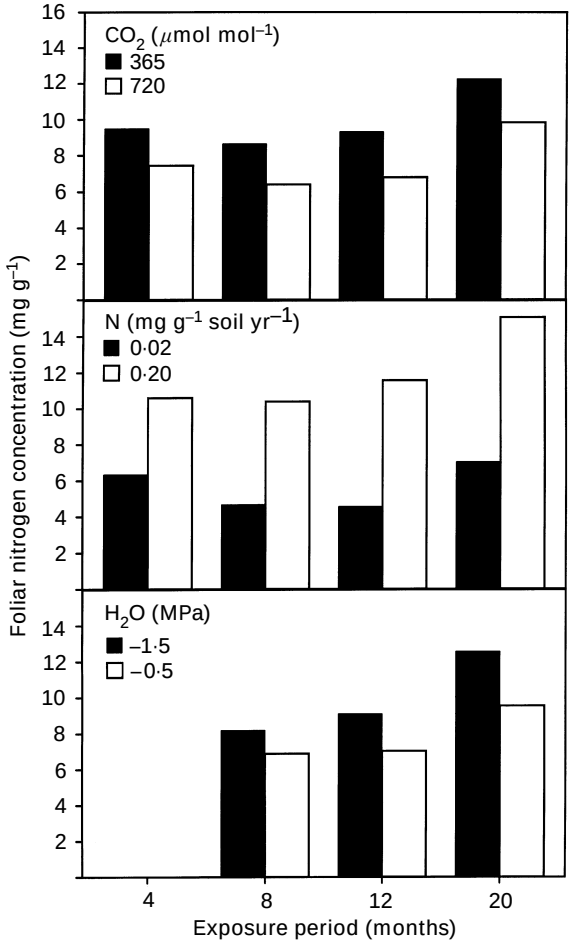

Figure 1. Foliar nitrogen concentrations for the CO_2, N, and water treatments at each of the four sampling periods (months of CO_2 exposure). In all cases, differences between treatments were significant at $P < 0{\cdot}01$.

seedlings had higher values only at high N fertility (Table 2).

DISCUSSION

Any change in a plant's environment might affect allocation patterns and alter mycorrhizal colonization and/or development within the plant root system (Andersen & Rygiewicz, 1991). Establishment of mycorrhizal infection is dependent upon availability of C in and around plant roots (Hacskaylo, 1973). Elevated atmospheric CO_2 often results in increased below-ground allocation of C (Rogers *et al*., 1994). Low levels of soil N can also increase C allocation to plant roots as a result of increased demand (Andersen & Rygiewicz, 1991; Green, Mitchell & Gjerstad, 1994; Mooney, Fichtner & Schulze, 1995). We have previously reported that partitioning to below-ground tissues (percentage of total biomass) and root to shoot ratio (R:S) of longleaf pine in this experiment were increased by elevated CO_2, low N, and adequate water (Prior *et al*., 1997). The present study documents similar response patterns for the percentage of ectomycorrhizal short roots and the number of ectomycorrhizas per unit root length for these same seedlings. The lower rates of mycorrhizal colonization at high N fertility (Marx, Hatch & Mendicino, 1997; Reid *et al*., 1983) and water stress (Worley & Hacskaylo, 1959; Mexal & Reid, 1973; Meyer, 1974) are consistent with reported trends. Increases in mycorrhizal colonization at elevated CO_2, either per unit root length (Norby *et al*., 1987; O'Neill *et al*., 1987; Monz *et al*., 1994) or on a whole-plant basis (Norby *et al*., 1986; O'Neill *et al*., 1991; Runion *et al*., 1994), have also been previously reported. The similar patterns for below-ground C allocation (Prior *et al*., 1997) and mycorrhizal response to soil resources and atmospheric CO_2 observed in this study suggested that sink/source relationships are a major factor regulating mycorrhizal development in high CO_2 environments. That is, when photosynthate supply was not source-limited (elevated CO_2), plants altered allocation to soil-resource acquisition (i.e. N or water) by investing in roots and mycorrhizas. Conversely, when N supply was not limiting, plants shifted allocation above-ground (presumably to increase acquisition of light for photosynthesis) resulting in lower levels of mycorrhizal colonization. Similarly, when tissue N concentrations were low (elevated CO_2, low soil N and adequate water) plants shifted allocation to those organs involved in N acquisition (i.e. mycorrhiza), as evidenced by the significant negative correlations between tissue N concentration and percentage or number of ectomycorrhizas.

Once established, mycorrhizal fungi require C from their host to build and maintain biomass and, to reproduce. This creates a demand for below-ground C allocation and under some stress conditions, the requirement for host C by mycorrhizal fungi has resulted in plant growth depression (Peng *et al*., 1993). Mycorrhiza can stimulate a host plant to allocate more C below-ground (Meyer, 1974) and can represent a significant C cost to the host, with estimates ranging from 4 to 17% of fixed C (Paul & Kucey, 1981; Rygiewicz & Andersen, 1994). In this study, although both above- and below-ground biomass were increased by higher levels of resource availability, the relative increase was greater below-ground at elevated CO_2 and high N (Prior *et al*., 1997). The increase in below-ground biomass was reflected in greater fine-root length and number of ectomycorrhizas per seedling at elevated CO_2 and adequate water; effects of N on ectomycorrhizas varied during the course of the experiment. Early in the study, even though root length was lower at low N, increased mycorrhizal colonization led to an increase in total number of mycorrhizas per seedling; after the 8-month harvest, greater root length under high N was the dominating factor, and resulted in greater number of ectomycorrhizas. This suggests

that, on the functionally significant scale of the whole plant, adequate nutrition resulted in more mycorrhizas in total, even though high levels of soil N suppressed mycorrhizal colonization.

Marx *et al*. (1977) also found that high levels of soil fertility reduced ectomycorrhizal infection, which they associated with lower levels of sucrose in pine roots. However, Lewis *et al*. (1994) found no significant correlation between carbohydrate concentration (either total sugars or starch) and mycorrhizal development on loblolly pine seedlings. The positive correlations between below-ground starch concentration and percentage or number of ectomycorrhizas observed in this study reflect overall sink/source trends of the host, while the negative correlation between mycorrhiza and steady-state soluble carbohydrate concentration might reflect changes in demand and use by the fungal symbiont (Hacskaylo, 1973). Understanding sink/source relationships, particularly quantifying the flux of C between above- and below-ground plant tissues, might give a greater understanding of mechanisms regulating mycorrhizal response in elevated-CO_2 environments (Zak *et al*., 1993; Pregitzer *et al*., 1995).

In our study, water stress resulted in less root length and fewer ectomycorrhizas. Feil, Kottke & Oberwinkler (1988) reported that drought stress increased branching density of Norway spruce (*Picea abies*) trees, which might explain the reduced root lengths observed in our study; however, they further stated that this increased branching was optimal for mycorrhizal development following rewetting, which was not seen here. Since water stress can result in decreased stomatal conductance and net photosynthesis (Parke *et al*., 1983; Augé *et al*., 1987), it is possible that insufficient photosynthate was available for mycorrhizal colonization and development.

In this study, the main treatment effects on both below-ground C allocation and mycorrhizal development were stronger (i.e. more significant) and more consistent than were interactions among these main treatments. However, interactions of N supply with CO_2 and water treatments did influence mycorrhizal development. Generally, elevated CO_2 or adequate water tended to result in increased ectomycorrhizal infection only at high soil N, possible as a result of interactions of soil N with CO_2 (Griffin, Thomas & Strain, 1993; Tissue, Thomas & Strain, 1993) or water (Green & Mitchell, 1992) determining source strength.

In addition to the influence of CO_2, N and water on sink/source relationships, variability in host/fungus systems and in other experimental conditions could explain some of the inconsistencies reported in the literature in mycorrhizal response to CO_2. It is also likely that differences in techniques might result in variation in measured responses (Grand & Harvey, 1982; Lewis *et al*., 1994). Differences in quantification might also explain the general trend for ectomycorrhizas to respond positively to higher levels of atmospheric CO_2 whereas VAM show less response (O'Neill, 1994). In general, VAM quantification is more time-consuming in that it requires clearing and staining procedures (ectomycorrhiza require no preparation) and use of a compound microscope (ectomycorrhiza can be assessed using a dissecting microscope). These factors have tended to result in VAM evaluations which are less quantitative, i.e. only the percentage of infection has been assessed in most CO_2-response studies (O'Neill *et al*., 1991; Monz *et al*., 1994; Runion *et al*., 1994). More detailed examination (e.g. proportion of root length infected and numbers of vesicles and arbuscules) would provide greater insights into VAM effects on plants and effects of the environment on VAM; however, collecting these data will require large amounts of time.

Meier (1991) suggested that qualitative (morphotype) assessment of ectomycorrhiza might provide insight to define more subtle plant responses to environmental stresses through shifts in the populations of the fungal symbionts. In this study, no differences in morphotype response were detected, which might indicate that different fungal symbionts responded similarly to treatment variables. However, this uniform response might indicate that the various morphotypes represent different developmental stages of a single symbiont. This latter possibility is supported in that only basidiocarps of *Thelephora terrestris* were observed and these were abundant at the time of planting and at each harvest.

Ectomycorrhizal development within host root systems is known to vary over time (Grand & Harvey, 1982; O'Neill *et al*., 1987; Andersen & Rygiewicz, 1991), possibly because production of new roots and colonization by ectomycorrhizal fungi are not necessarily concurrent processes. The reduction in the percentage of ectomycorrhizal short roots and number of ectomycorrhiza per metre of fine root observed between the 12- and 20-month harvests (March–November 1994) might have resulted from an inability of infection by ectomycorrhizal fungi to keep pace with root production. This is supported by the large increase in fine-root length during this period as well as the fact that ectomycorrhizal variables at the final harvest were similar to those at the first harvest, probably another period of rapid root proliferation.

In conclusion, we report that mycorrhizal development on longleaf pine seedlings was enhanced by elevated CO_2, low soil N and adequate water. These main effects tended to be strong and stable over time, while interactions were more variable and less significant. Treatment effects on mycorrhizal development were consistent with observed and

reported influences of these variables on sink/source relationships. Thus, understanding mechanistic controls on sink/source relationships under CO_2-enrichment might enable a greater understanding of potential shifts in symbiotic relationships as a result of atmospheric change.

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